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Signal Transduction: Bacterial Thermometer Works by Measuring Membrane Thickness

Cells detect external chemical stimuli by directly binding a signaling molecule, but the strategies used by cells to detect and respond to non-chemical cues have been mysterious. Recent work suggests that a bacterial protein detects changes in environmental temperature by physically measuring membrane thickness.

Kumaran S. Ramamurthi

The ability to sense environmental conditions and mount an adaptive transcriptional response is conserved in all domains of life. Moreover, reversible protein phosphorylation is a widespread mechanistic strategy for the intracellular transduction of extracellular signals. In bacteria, a paradigmatic signal transduction mechanism (appropriately termed a 'two-component system') utilizes just two proteins [1]. The first is a transmembrane protein called a 'sensor kinase' that detects an environmental signal and transmits the information to the interior of the cell. Sensor kinases have two functional domains: a signal recognition domain (often extracellular) that may physically bind to a ligand, and an intracellular autokinase domain that contains a characteristic histidine residue [2]. Detection of an environmental signal activates the autokinase domain and results in phosphorylation of this histidine. The second component of the system is canonically a soluble transcription factor called a 'response regulator', which is activated when it accepts the phosphoryl group from its cognate sensor kinase. The response regulator then typically activates the transcription of appropriate genes that respond to the extracellular stress. In this model, chemical factors, such as salts, nutrients, and signaling peptides, may be detected by direct binding of these ligands to the sensor kinase, leading to its activation by autophosphorylation (Figure 1A). How, though, can a sensor kinase detect a more abstract, non-chemical environmental cue such as temperature?

In this issue, Cybulski *et al.* [3] report their studies on the *Bacillus subtilis* sensor kinase DesK, which is activated in response to reduced temperature [4]. Specifically, the authors wondered if there is a physical feature of the cell which changes in response to fluctuations in temperature that DesK may physically detect and exploit as an indirect measure for temperature. The plasma membrane of the bacterium was a key candidate for harboring this physical feature for two reasons. First, DesK is a polytopic membrane protein [4]. Second, previous studies had demonstrated that increasing the fluidity of the plasma membrane by increasing the incorporation of branched-chain fatty acids decreased the activation of DesK, suggesting that DesK directly responds to a physical property of the plasma membrane [5]. Now Cybulski *et al.* [3] propose a model in which DesK detects an increase in the *thickness* of the plasma membrane upon a drop in temperature, resulting in DesK activation.

The authors began by performing a systematic deletion analysis of the unwieldy five-pass integral membrane protein and discovered that deletion of just the first transmembrane region (TM1) abolished the ability of DesK to respond to lower temperature and resulted in a constitutively active protein, suggesting that TM1 harbored a temperature-sensing motif. Meanwhile, previous structural studies had indicated that the last transmembrane helix of DesK (TM5) is attached to the autokinase domain through a two helical coiled-coil motif that appeared to be critical for regulation of DesK activity [6]. The authors envisioned a model in which TM1 would detect a drop in temperature and transmit that information to TM5, which would then activate DesK. They therefore created a chimeric transmembrane region, consisting of amino-terminal residues of TM1 and carboxy-terminal residues of TM5, and fused it to the autokinase domain. Remarkably, this simplified DesK, harboring a single engineered transmembrane segment (the minimal thermo-sensor), worked almost as well as wild-type DesK harboring five membrane-spanning helices.

Curiously, the amino terminus of the minimal thermo-sensor contained a cluster of hydrophilic amino acids near the lipid–water interface. At high temperatures, when lipids are disordered and the bilayer is thinner, the authors reasoned that these residues could "float" on the membrane surface, like a buoy, while being tethered to the membrane itself by the hydrophobic residues of the transmembrane region (Figure 1B). In this conformation, the authors supposed that DesK autokinase activity would be low. A drop in temperature, conversely, would increase lipid ordering, resulting in a thicker plasma membrane. In this scenario, the authors predicted that these hydrophilic residues would become forcibly buried into the hydrophobic lipid bilayer, resulting in a conformational change that would activate the autokinase activity of DesK. To test this, the authors lengthened the transmembrane region of the thermo-sensor so that the 'buoy' was farther away from the surface of the membrane and would not be pulled into the bilayer when the membrane

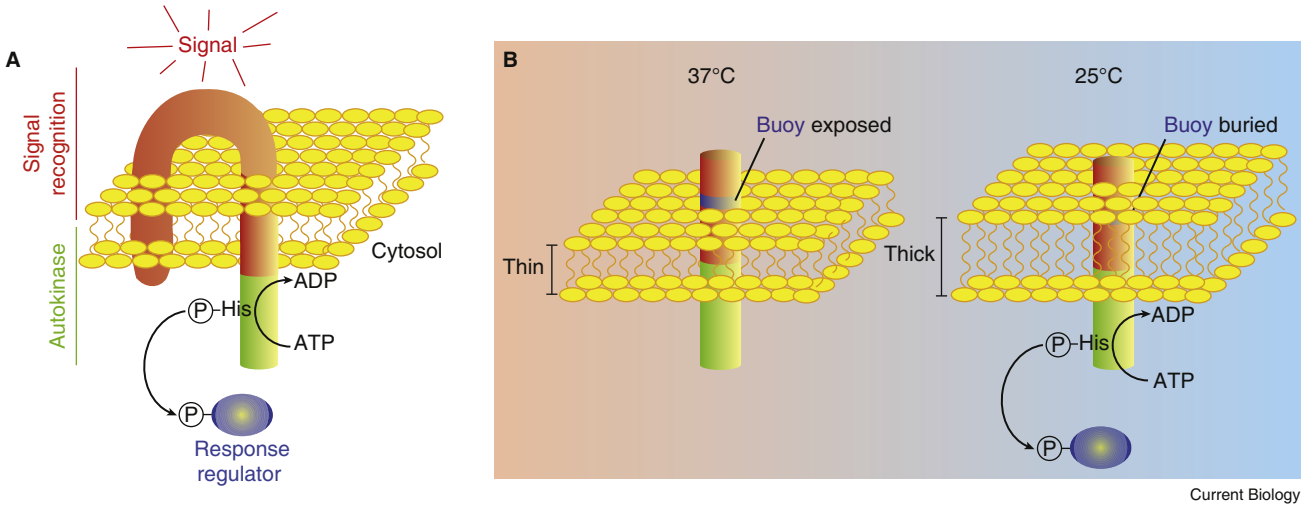

Figure 1. Temperature sensing by measuring membrane thickness.

(A) Depiction of a canonical bacterial two-component regulatory system. A transmembrane sensor kinase is shown with an extracytoplasmic signal recognition domain (red) and an intracellular autokinase domain (green). Sensor kinases often have multiple membrane-spanning domains, but, for simplicity, only two membrane-spanning domains are depicted. Detection of an activating signal results in autophosphorylation of a characteristic histidine residue in the autokinase domain and subsequent transfer of the phosphoryl group onto a cognate response regulator (blue), typically a transcription factor. (B) Depiction of an engineered minimal signal-sensing domain of DesK, a sensor kinase in *B. subtilis* that detects changes in environmental temperature. The minimal thermo-sensor harbors a cluster of hydrophilic amino acids (blue ring) near its amino terminus (extracytoplasmic) that floats like a buoy near the lipid–water interface at high temperatures when the membrane is thinner (left). At lower temperatures (right), an increase in lipid ordering results in a thicker membrane, forcing the 'buoy' into the hydrophobic lipid bilayer, thereby activating the autokinase domain. DesK therefore responds to changes in temperature by directly measuring membrane thickness.

thickened. As expected, the lengthened version of the minimal thermo-sensor was unable to activate DesK at lower temperature; that is, it was unable to detect the thickening of the membrane. In complementary biochemical studies, the authors reconstituted the form of DesK that harbors the minimal thermo-sensor into membrane vesicles made of phosopholipids of varying fatty acyl chain length (and therefore varying membrane thicknesses). As predicted, the autokinase activity of DesK was higher at lower temperature when DesK was reconstituted into vesicles made from long chain phospholipids (thick membranes). In the presence of short chain phospholipids (thin membranes), DesK autokinase activity was diminished, presumably because the 'buoy' was too far away from the surface of the membrane to accurately measure its expansion and contraction. Taken together, the data led the authors to suggest a model in which the cluster of hydrophilic residues of DesK that forms the buoy actually functions like the end of a ruler that physically measures membrane thickness as an indication of environmental temperature.

As the authors mentioned, DesK now joins a list of proteins of diverse cellular functions that harbor domains that act as molecular rulers. Tail lengths of bacteriophages and needle lengths of bacterial type III protein secretion machines are precisely determined by a single molecular ruler protein: deletion or addition of amino-acid residues to the middle of these proteins results in the proportional decrease or increase in the length of the tail or needle [7,8]. In yeast, during the biosynthesis of very long chain fatty acids, shortening the distance between a particular amino acid residue and the active site of a component of the biosynthetic machinery results in the production of fatty acids with shorter chain lengths [9]. Beyond rulers, several proteins of diverse structure and function have even been described that are equipped with 'molecular protractors' that measure degrees of membrane curvature when carrying out their function [10–12].

A long-standing challenge in studying two-component regulatory systems has been to understand the input signals that actually activate sensor kinases. Could *physical* cues, like membrane thickness, curvature, viscosity, or tension, be activators of other transmembrane sensor kinases for which chemical activating signals have been difficult to identify? Although the exploitation of tools such as molecular rulers and protractors has more often been described for morphogenetic processes, such as the assembly of structures and the localization of proteins, perhaps future studies will reveal that other systems, like gene regulatory pathways, also often measure and respond not just to chemical signals, but to physical cues.

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Memory: Reconsolidation Allows Modification of Motor Memories

A recent study using non-invasive transcranial magnetic stimulation has revealed how specific brain processing during memory reactivation makes possible the modification of existing memories that is required for motor learning.

Niels Birbaumer

Memory research represents one of the most challenging and exciting areas of basic and systems neuroscience. Our brain constantly encodes the features of the surrounding environment, a critical function for our everyday survival as well as for learning leading to successful interactions with the external world. Such interactions require constant updates and 'tune ups' of the brain's internal representations or memories.

In everyday life, memories can be automatically modified in healthy human beings. For example, we barely notice very slow changes in friends or family members whom we see everyday (as opposed to changes in people whom we haven't seen for longer periods of time). Our brain updates the internal representation of these memories probably every time we see that person again. Thus, changes are often unnoticeable to us.

When learning to perform a motor task in everyday life, the need to repeatedly update the memory trace becomes even more critical because most skills are acquired over time. Surprisingly, the mechanisms and the cerebral regions that mediate the human brain's ability to modify existing memories have still not been clearly identified. Animal researchers have used invasive approaches to inhibit specific brain areas, revealing the mechanisms underlying modification of existing memories following their reactivation during recall [1–4]. Such approaches are not possible in human research. In this issue of *Current Biology*, Censor *et al.* [5] report how they used non-invasive brain stimulation — specifically, repetitive transcranial magnetic stimulation (rTMS) — to virtually 'knock out' focal human brain areas during the susceptible time frame of memory reactivation, thereby unveiling human brain processes that allow modification of reactivated existing memories.

Transcranial magnetic stimulation operates by inducing a magnetic field, which results in flow of currents parallel to the stimulating coil and neural activation in the targeted brain area [6,7]. Generally, low-frequency rTMS (usually 1 Hz) induces inhibitory effects allowing a reversible 'virtual lesion' in focal brain areas [8]. This approach, somewhat resembling the 'gene knockout' technique of genetic research (though the direct effects induced by rTMS are temporal and reversible), makes it possible to study the functional role of the targeted brain area in spatial and temporal domains of learning and memory processes.

In this new study [5], subjects performed a sequential finger tapping motor memory task on three separate days. When receiving no stimulation, subjects improved from day 1 to day 2, and continued to improve when tested on day 3 [5,9]. Here, subjects showed off-line performance gains from day 1 to day 2, pointing to efficient consolidation of the motor memory as reported in previous studies [9,10]. Following testing on day 2, subjects received 15 minutes of 1 Hz rTMS to primary motor cortex (M1), while performing additional trials of the task during the stimulation period in order to reactivate the memory trace as required for reconsolidation [1–4]. This disruption of M1 activity during memory reactivation blocked further memory modification, with subjects showing no significant memory gains on day 3.

Censor *et al.* [5] used conventional physiological measurements in order to disturb M1 function in its appropriate location and intensity of stimulation and, furthermore, used a stereotactic brain navigation system and each subject's magnetic resonance image (MRI) to localize the stimulating coil online. In order to further control for the anatomical specificity of the rTMS effects, the authors conducted a similar experiment in which rTMS was applied to a control vertex position with the same stimulation parameters, with results showing that stimulation of a brain region different from M1 did not block memory modification. In an additional experiment, they showed that disruption of manual execution of the motor actions *per se* with peripheral nerve stimulation at the wrist also does not block memory modification. These experiments show that specific disruption of M1 processing during memory reactivation blocks memory modification.

Censor *et al.* [5] conclude by suggesting a model for human memory modification, susceptible to future further testing (Figure 1). The significance of this model lies in the fact that it differentiates between what the authors refer to as 'memory storage domains', allowing novel characterization of the actual human brain areas involved in modification of existing memories. According to the model, when the memory is